

Durham Research Online

Deposited in DRO:

21 July 2020

Version of attached file:

Accepted Version

Peer-review status of attached file:

Peer-reviewed

Citation for published item:

Romero, Isabel and Martinez-Medina, Margarita and Camprubí-Font, Carla and Bennour, Ines and Moreno, David and Martínez-Martínez, Luis and Teixidor, Francesc and Fox, Mark A. and Viñas, Clara (2020) 'Metallacarborane assemblies as effective antimicrobial agents, including a highly potent anti-MRSA agent.', *Organometallics*, 39 (23). pp. 4253-4264.

Further information on publisher's website:

<https://doi.org/10.1021/acs.organomet.0c00315>

Publisher's copyright statement:

This document is the Accepted Manuscript version of a Published Work that appeared in final form in *Organometallics* copyright © American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see <https://doi.org/10.1021/acs.organomet.0c00315>

Additional information:

Use policy

The full-text may be used and/or reproduced, and given to third parties in any format or medium, without prior permission or charge, for personal research or study, educational, or not-for-profit purposes provided that:

- a full bibliographic reference is made to the original source
- a [link](#) is made to the metadata record in DRO
- the full-text is not changed in any way

The full-text must not be sold in any format or medium without the formal permission of the copyright holders.

Please consult the [full DRO policy](#) for further details.

Metallacarborane assemblies as effective antimicrobial agents including a highly potent anti-MRSA agent.

Isabel Romero García,^a Margarita Martinez-Medina,^b Carla Camprubí-Font,^b
Ines Bennour,^c David Moreno,^a Luis Martínez-Martínez,^d Francesc Teixidor,^c
Mark A. Fox,^e Clara Viñas.^{c,*}

^a Departament de Química and Serveis Tècnics de Recerca, Universitat de Girona, C/ M.
Aurèlia Campmany, 69, E-17003 Girona, Spain

^b Microbiology of the Intestinal Disease group, Biology Department of
Biology, Universitat de Girona, 17003 Girona, Spain.

^c Institut de Ciència de Materials de Barcelona, Consejo Superior de Investigaciones
Científicas, Campus Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain Fax:
(internat.) + 34-935805729

^d Unidad de Gestión Clínica de Microbiología, Hospital Universitario Reina Sofía,
IMIBIC, Universidad de Cordoba, Cordoba, Spain.

^e Chemistry Department, Durham University, South Road, Durham DH1 3LE, U.K.

Corresponding author: Prof. Clara Viñas clara@icmab.es

"Dedicated to Prof. Alan Welch, a great scientist, a great person and a great friend on
the occasion of his retirement from Heriot-Watt University."

Abstract

The salts, Na[ROC(O)Ph], Na[1-ROC(O)-1,12-C₂B₁₀H₁₁], K₂[1,4-(ROC(O))₂-C₆H₄] and K₂[1,12-(ROC(O))₂-1,12-C₂B₁₀H₁₀] where R is the cobaltabis(dicarbollide)-diethylene glycol group [3,3'-Co(8-(OCH₂CH₂OCH₂CH₂)-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)]⁻, were synthesized from the corresponding carboxylate salt and the zwitterion [3,3'-Co(8-(CH₂CH₂O)₂-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)]⁻. The dianion in K₂[1,12-(ROC(O))₂-1,12-C₂B₁₀H₁₀] showed at least one K⁺ cation to be tightly bound to the dianion via K⁺⋯O and K⁺⋯H-B interactions with both *para*-carborane and cobaltabis(dicarbollide) clusters based on NMR and MS data and supported by hybrid-DFT computations. Evaluation of the antimicrobial properties of these compounds revealed all salts to be highly effective antibacterial agents for four Gram-positive bacteria strains (standard minimum inhibitory concentration, MIC, of 1 mg/L for Na[ROC(O)Ph]; Na[**3**]) and antifungal agents for three *Candida albicans* strains (MIC 4 mg/L for the salts K₂[**4**] and K₂[**6**]). One of the four Gram-positive bacteria strains tested was a life-threatening superbug methicillin-resistant *Staphylococcus aureus* (MRSA) isolate, which is resistant to many commercial antimicrobial drugs. The cobaltabis(dicarbollide) derivative, Na[**3**], has a remarkable inhibitory effect on the MRSA strain with a MIC of only 1 mg/L and a minimum bactericidal concentration (MBC) of 2 mg/L thus suggesting its potential as an antibacterial agent against MRSA.

Keywords: antibacterial, antifungal, metallacarborane, cobaltabis(dicarbollide), MRSA.

Introduction

Most of the FDA approved anticancer and antibacterial drugs are purely organic molecules, which can incorporate nitrogen, oxygen, and halogens - all of them right-hand neighbors of carbon. The determination of vitamin B₁₂ (cyano-cobalamin) structure¹ as well as the successful introduction of cisplatin as an anticancer drug² in the early 1980s opened the door to potential applications of organometallic complexes in medicine.³

Boron is located on the left side of the carbon in the Periodic Table. Boron and carbon are elements that have the property to build molecules of unlimited size by covalent self-bonding. The twelve-vertex C₂B₁₀H₁₂ carboranes, *ortho*-, *meta*-, or *para*-isomers (Chart 1), rank among the most chemical and biological stable molecular compounds known.^{4,5} Metallacarboranes are derivatives of carboranes with metal atoms in the cluster framework.⁴ The metallabisdicarbollide [3,3'-Co(1,2-C₂B₉H₁₁)₂]⁻ [**1**]⁻ (Chart 1), in which the metal ion is sandwiched between two [C₂B₉H₁₁]²⁻ ligands, is the most studied icosahedral metallacarborane.⁶ The anion, [**1**]⁻, is thermally and chemically stable, its negative charge is spread all over the molecule,^{4,7} possesses hydrogen and dihydrogen bonds (C_c-H...O and C_c-H...H-B, respectively; C_c = cluster carbon atom) capabilities which have been present in self-assemblies,⁸ water solubility,⁹ micelle and vesicle formation,¹⁰ and has derivatization capacity similar or superior to organic compounds.^{4,5}

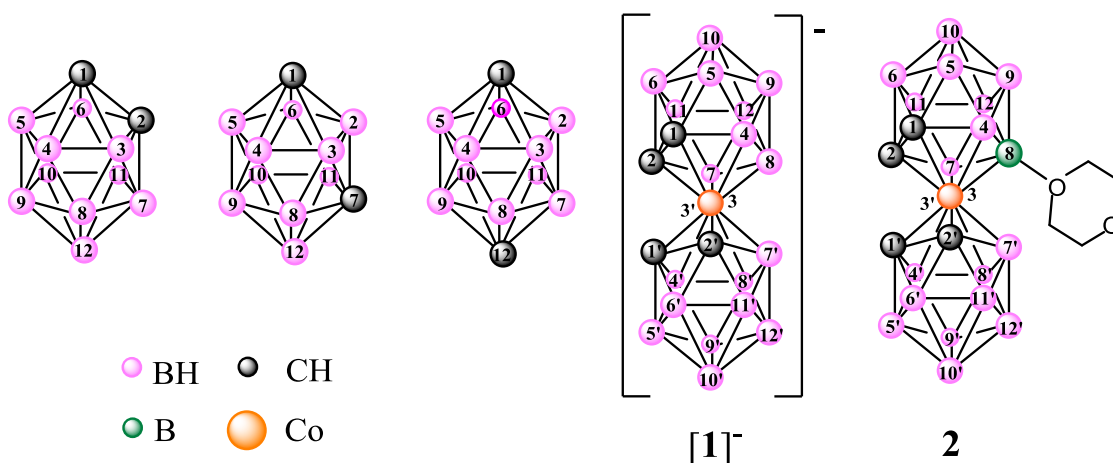


Chart 1. Representation and numbering of icosahedral carboranes (*ortho*-, *meta*-, or *para*- isomers) and the organometallic compounds, anion $[1]^-$ and zwitterion **2**.

The metallacarborane $[1]^-$ anion is a highly compact Greek letter θ shaped molecule ready for stepwise substitutions at boron atoms.⁶ The derivatization of the cobaltabis(dicarbollide) through the easy synthesis of the zwitterion 8-dioxanate [3,3'-Co(8-(CH₂CH₂O)₂-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)], **2** in Chart 1, in a high yield^{11,12} opened the way for new applications of this organometallic anion. The mononuclear complex **2** is susceptible to nucleophilic attack on the positively charged oxygen atom, e.g. by pyrrolides,¹³ imide, cyanide or amines,¹⁴ phenolate, dialkyl or diarylphosphite,¹⁵ N-alkylcarbamoyldiphenylphosphine oxides,¹⁶ alkoxides,^{12,17} nucleosides,¹⁸ and carboranyl anions¹⁹ yielding many anionic diethylene glycol cobaltabisdicarbollide species formed by the opening of the dioxane ring.

Boron compounds (e.g. boronic acids, boron heterocycles, carboranes) in medicine are usually associated with the properties of boron as element within deltahedral species primarily to exploit the ¹⁰B isotope for neutron capture which led to the development of the Boron Neutron Capture Therapy (BNCT) as anticancer agents.²⁰ Some boron compounds can also show promise as effective antibacterial and antifungal

agents.^{21,22} In 2013, some of us screened derivatives of [1]⁻ to evaluate their putative antimicrobial properties against 16 pathogenic Gram-positive and Gram-negative bacterial strains and 3 strains of fungal *Candida spp.* where salts **A** and **B** in Chart 2 were the most effective antimicrobial agents against all strains.²³ A derivative of [1]⁻, [3,3'-Co(8-CH₃CH₂O-1,2-C₂B₉H₁₀)(8'-I-1',2'-C₂B₉H₁₀)]⁻, **C**, was reported by Wang and co-workers to possess a high bacteria-killing efficiency with eradication of all TSA MRSA cells within 30 min.²⁴ However, this compound was not effective for other Gram-positive and Gram-negative bacteria tested. Recently, Šícha and co-workers reported that the salt Na[1] and zwitterions such as **D**, **E** and **F** were effective against Gram-positive bacteria and filamentous fungi but not Gram-negative bacteria and *Candida spp.* (yeast fungi).^{25,26}

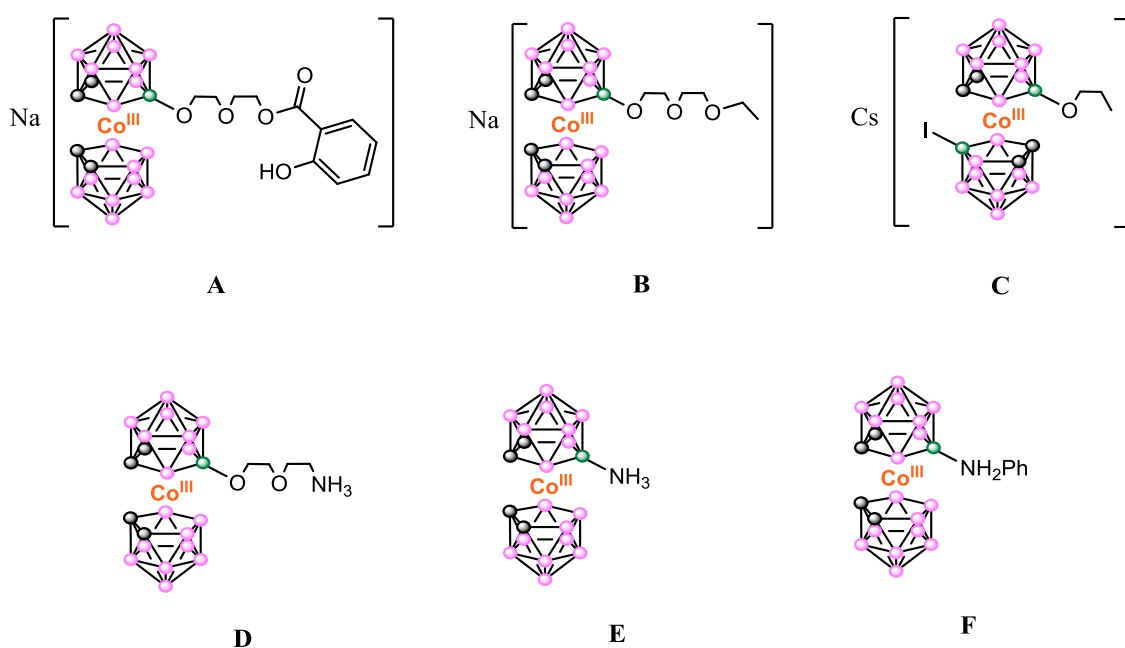


Chart 2. Known cobaltabis(dicarbollide) derivatives with effective antimicrobial properties. Compounds **A-C** are anionic and **D-F** are zwitterion.

Medical treatment for some microbial illnesses is complicated nowadays by the appearance of new multi-resistant strains, and therefore, a new class of highly selective antimicrobial boron- containing compounds could be of importance.²⁷ Following our studies on metallacarboranes' direct substitution, we report herein high yield syntheses

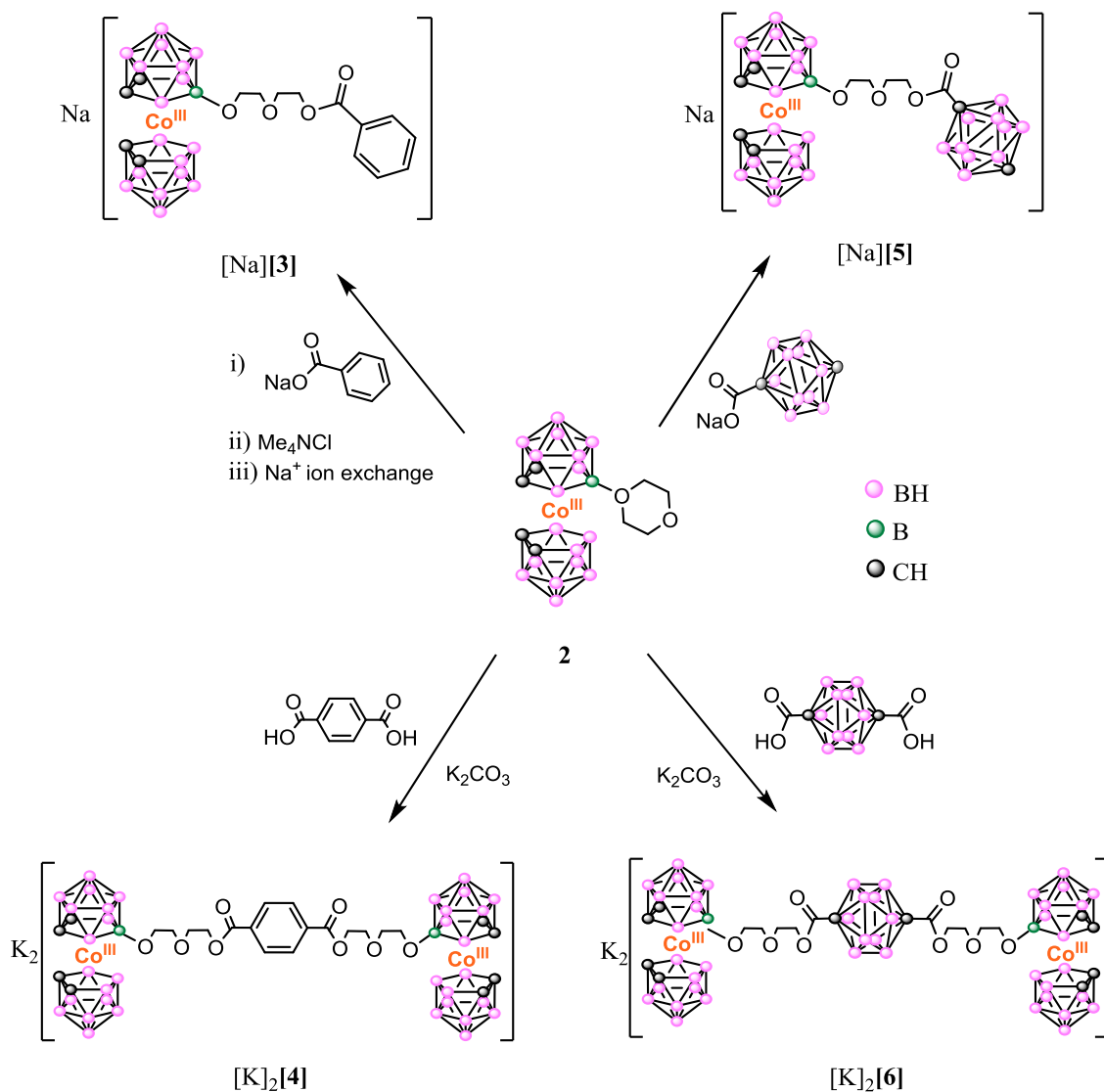
of polyanionic species incorporating the cobaltabis(dicarbollide) anion as novel high-boron content molecules with enhanced water solubility. Two of these new assemblies include the *para*-carborane cluster and the others the *para*-phenyl group. These assemblies were tested here with 4 Gram-positive bacteria, 5 Gram-negative bacteria and 3 *Candida albicans* strains that were previously responsible for human infections, and all were found to be effective against Gram-positive bacteria and fungi.

Results and discussion

Synthesis and characterization

The synthetic strategy for the preparation of the Co(III) compounds, Na[**3**], K₂[**4**], Na[**5**] and K₂[**6**], is outlined in Scheme 1. These salts were obtained by the ring-opening reaction of cyclic oxonium [3,3'-Co(8-(CH₂CH₂O)₂-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)] zwitterion **2**,^{11,12} with organic ([C₆H₅COO]⁻ and [C₆H₄-1,4-(COO)₂]²⁻) and inorganic carboxylates ([1-COO-1,12-C₂B₁₀H₁₁]⁻ and [1,12-(COO)₂-1,12-C₂B₁₀H₁₀]²⁻), as nucleophiles. Sodium salts of the monoacid ligands, benzoic and 1-COOH-1,12-C₂B₁₀H₁₁ acids, provided nucleophiles on **2** to produce the monoanionic Na[3,3'-Co(8-O(CH₂CH₂O)₂C(O)C₆H₅-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)], Na[**3**], and Na[3,3'-Co(8-O(CH₂CH₂O)₂C(O)-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)-1,12-C₂B₁₀H₁₁], Na[**5**], respectively. The potassium salt, generated *in situ* from terephthalic acid and potassium carbonate, contained the nucleophile used to attack **2** to afford the di-anionic compound K₂[1'',4''-{3,3'-Co(8-O(CH₂CH₂O)₂CO-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)}₂-C₆H₄] K₂[**4**]. Likewise K₂[1'',12''-{3,3'-Co(8-O(CH₂CH₂O)₂CO-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)}₂-1'',12''-C₂B₁₀H₁₀] K₂[**6**], was formed from the salt generated from K₂CO₃ and 1,12-C₂B₁₀H₁₀-1,12-(COOH)₂ with the zwitterion **2**. After stirring overnight at ambient temperatures, the

anionic compounds were isolated as orange solids by evaporation of the solvent. Complex $K_2[4]$ was further separated and purified through column chromatography in silica.



Scheme 1. Synthetic routes used in this work.

The isolated compounds were characterized by FT-IR, NMR (1H , ^{11}B , $^{13}C\{^1H\}$ nuclei) spectroscopies, and mass spectrometry. IR spectra of these new salts show vibrations around $2960\text{--}2860\text{ cm}^{-1}$ assigned to ν_{C-H} stretching modes for the chain of the dioxane opened ring while the $C_c\text{--}H$ stretches of the $1,12\text{-}C_2B_{10}H_{11}$ unit in $Na[5]$ and cobaltabis(dicarbollide) units appear at 3048 cm^{-1} . Significant bands around 2530 cm^{-1}

correspond to the $\nu_{\text{B-H}}$ stretching modes for the B-H bonds of the boron cluster units. Strong bands are also observed at 1700 cm^{-1} and assigned to $\nu_{\text{C=O}}$ stretching band of the carboxyl group present in the compounds.

One-dimensional (1D) NMR spectra of all new salts were recorded in d_6 -acetone (see S.I.) and are consistent with the structures displayed in Scheme 1. Proton NMR peaks of B(8)-OCH_2^- , $-\text{CH}_2\text{OCH}_2^-$ and $-\text{CH}_2\text{-R}$ in all salts were observed in the range between 4.47 and 3.52 ppm. The presence of $\text{C}_c\text{-H}$ signals corresponding to the cobaltabis(dicarbollide) unit appear as a broad signal around 4.25 ppm. The salts, Na[3] and $\text{K}_2\text{[4]}$, exhibit signals in the aromatic region associated with the presence of aromatic phenyl rings. In the case of compound Na[5] , the resonance corresponding to $\text{C}_c\text{-H}$ of the $1,12\text{-C}_2\text{B}_{10}\text{H}_{11}$ moiety appears at 3.32 ppm.

The $^{11}\text{B}\{^1\text{H}\}$ NMR spectra of the four anions in the new salts featured an identical 1:1:1:1:2:2:4:2:2:1:1 pattern ranging from +25 to -30 ppm corresponding to the metallacarborane unit with values listed in Table 1. The resonance at the lowest field remains as a singlet in the ^{11}B NMR spectrum and corresponds to the B(8) substituted boron atom. As shown in Figure 1, anion $[\mathbf{5}]^-$ could be considered as the sum of the fragment I and fragment II. The schematic representation of the $^{11}\text{B}\{^1\text{H}\}$ NMR spectrum of the $[\mathbf{5}]^-$ consists of 13 peaks with a 1:1:1:1:2:2:4:5:5:2:2:1:1 intensity ratio; 11 resonances from the fragment I (1:1:1:1:2:2:4:2:2:1:1) plus 2 resonances (5:5) from the fragment II (Figure 2).

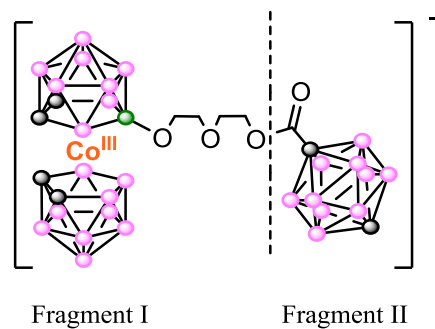


Figure 1. Notional fragmentation of the monoanion $[5]^-$.

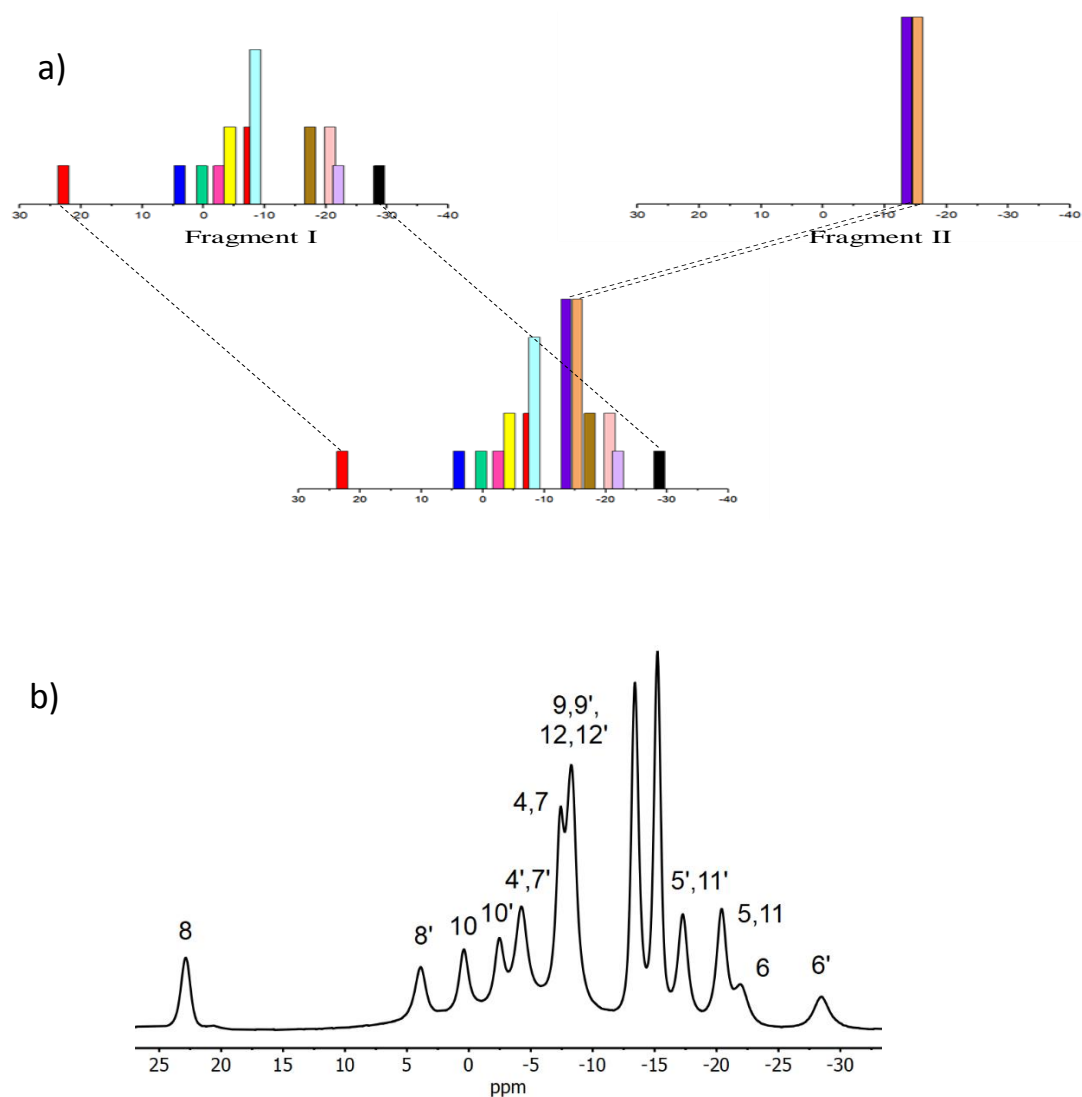


Figure 2. a) Schematic representation of $^{11}\text{B}\{^1\text{H}\}$ NMR spectrum of anion $[5]^-$ as the sum of the fragment I and II and b) experimental $^{11}\text{B}\{^1\text{H}\}$ NMR spectrum of $\text{Na}[5]$.

Table 1. $^{11}\text{B}\{^1\text{H}\}$ NMR chemical shifts (ppm) of the cobaltabis(dicarbollide) unit in compounds [3]–[6]²⁻.

B(8) values correspond to B-O resonances.

	B(8)	B(8')	B(10)	B(10')	B(4',7')	B(4,7)	B(9,9', 12,12')	B(5',11')	B(5,11)	B(6)	B(6')
[3] ⁻	23.0	4.0	0.5	-2.4	-4.1	-7.4	-8.3	-17.2	-20.3	-21.9	-28.5
[4] ²⁻	24.1	5.1	1.3	-1.6	-3.5	-6.5	-7.4	-16.5	-19.6	-20.9	-28.3
[5] ⁻	22.6	3.7	0.1	-2.8	-4.6	-7.8	-8.6	-17.7	-20.8	-22.3	-29.0
[6] ²⁻	24.2	5.1	1.5	-1.4	-3.2	-6.3	-7.2	-16.2	-19.4	-21.0	-27.6

The boron-decoupled proton $^1\text{H}\{^{11}\text{B}\}$ NMR spectra reveal broad BH peaks characteristic of the 8-substituted cobaltabis(dicarbollide) anion in all complexes. These peaks are usually in a 4:2:1:1:2:2:2:1 peak integral ratio and, in deuterated acetone, the shifts are in the 3.0-2.7 ppm region for 4:2:1 and in the 2.0 to 1.4 ppm range for 2:2:2:2:1. There is a peak of intensity 1 in the 2.4 ppm region for a 8-substituted cobaltabis(dicarbollide) anion which is in the same region expected for the B-H shift corresponding to the protons of the substituted *para*-carborane at the B2-B6 vertices. Figure 3 shows the 2.6 to 1.9 ppm region for the *para*-carborane precursors and the *para*-carborane salts, Na[5] and K₂[6]. There are two B-H peaks at 2.43 and 2.22 ppm with intensity ratio of 6:5 in Na[5] which is in accord with the two distinct set of BH peaks with 5:5 ratio plus a BH peak from the cobalt cluster anion. However, instead of the expected peak intensity ratio of 2:10 for K₂[6] with two BH protons from the cobaltabis(dicarbollide) and all 10 equivalent BH protons from the *para*-carborane cluster, there are three peaks at 2.49, 2.44 and 2.40 ppm with a 4:4:4 integral ratio. Peak fitting analysis suggests that the *para* carborane B-Hs are 4:2:4 with a broader peak of two B-Hs from the cobalt cluster at 2.44 ppm (Figure S33). These peaks indicate that not all protons at the *para*-carborane unit are equivalent in K₂[6] and there is likely to be at least one potassium cation in close interaction with the neutral *closo*-carboranyl unit in solution resulting in non-equivalent BH environments at the latter cluster.

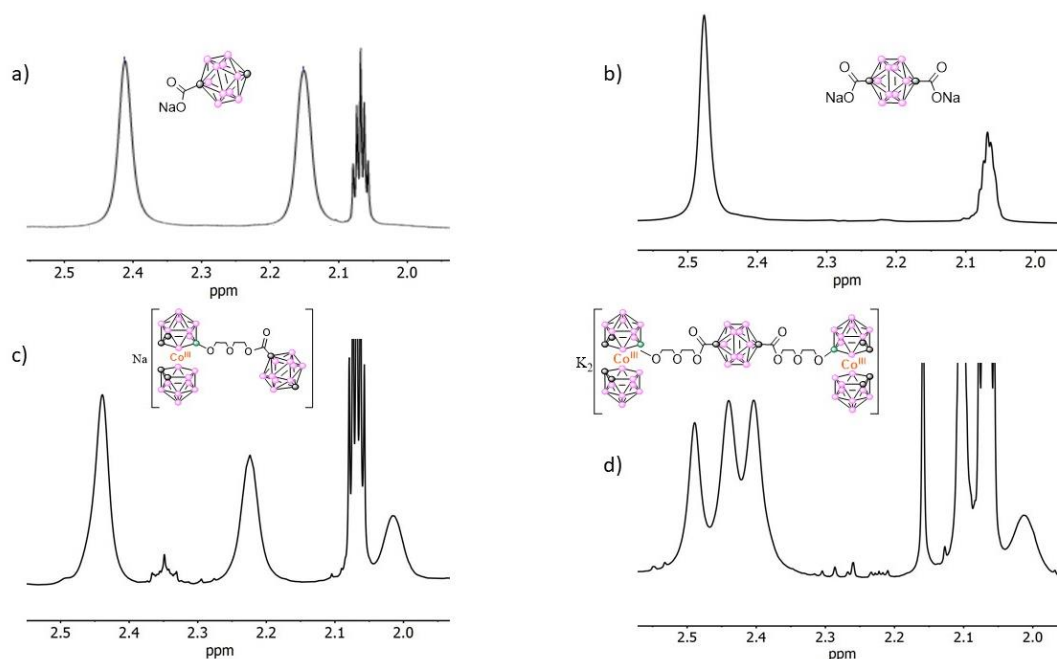


Figure 3. $^1\text{H}\{^{11}\text{B}\}$ NMR spectra of (a) $\text{Na}[1\text{-COO-}1,12\text{-C}_2\text{B}_{10}\text{H}_{11}]$, (b) $\text{Na}_2[1,12\text{-(COO)}_2\text{-}1,12\text{-C}_2\text{B}_{10}\text{H}_{10}]$, (c) $\text{Na}[\mathbf{5}]$ and (d) $\text{K}_2[\mathbf{6}]$ in $(\text{CD}_3)_2\text{CO}$ ($\text{CD}_3\text{COCHD}_2$ at 2.06 ppm and, if present, CH_3COCH_3 is at 2.09 ppm).

For monoanions $[\mathbf{3}]^-$ and $[\mathbf{5}]^-$, the ESI-MS spectra displayed peaks at m/z 532.2 and 598.4, respectively, that correspond in both cases to the molecular weight of the monoanionic species. In the case of $\text{K}_2[\mathbf{4}]$, the most intense peak corresponding to $M/2$ appears at m/z 493.2 with a separation between isotopic peaks distribution of 0.50 m/z units that unambiguously identifies the species as the di-anion fragment, with M as the molecular weight of the dianionic $[\mathbf{4}]^{2-}$ species. In addition, three peaks with a separation between isotopic peaks distribution of 1 m/z units at m/z 620.2, 428.2, and 383.2 correspond to the fragmentation of the dianionic $[\mathbf{4}]^{2-}$ species into monoanionic fragments $[3,3'\text{-Co(8-HOCH}_2\text{CH}_2\text{-OOC-C}_6\text{H}_4\text{-COO-(CH}_2\text{CH}_2\text{O)}_2\text{-}1,2\text{-C}_2\text{B}_9\text{H}_{10})(1,2\text{-C}_2\text{B}_9\text{H}_{11})]^-$, $[3,3'\text{-Co(8-(CH}_2\text{CH}_2\text{O)}_2\text{OH-}1,2\text{-C}_2\text{B}_9\text{H}_{10})(1,2\text{-C}_2\text{B}_9\text{H}_{11})]^-$, and $[3,3'\text{-Co(8-OCH}_2\text{CH}_2\text{OH-}1,2\text{-C}_2\text{B}_9\text{H}_{10})(1',2'\text{-C}_2\text{B}_9\text{H}_{11})]^-$, respectively (Figure 4).

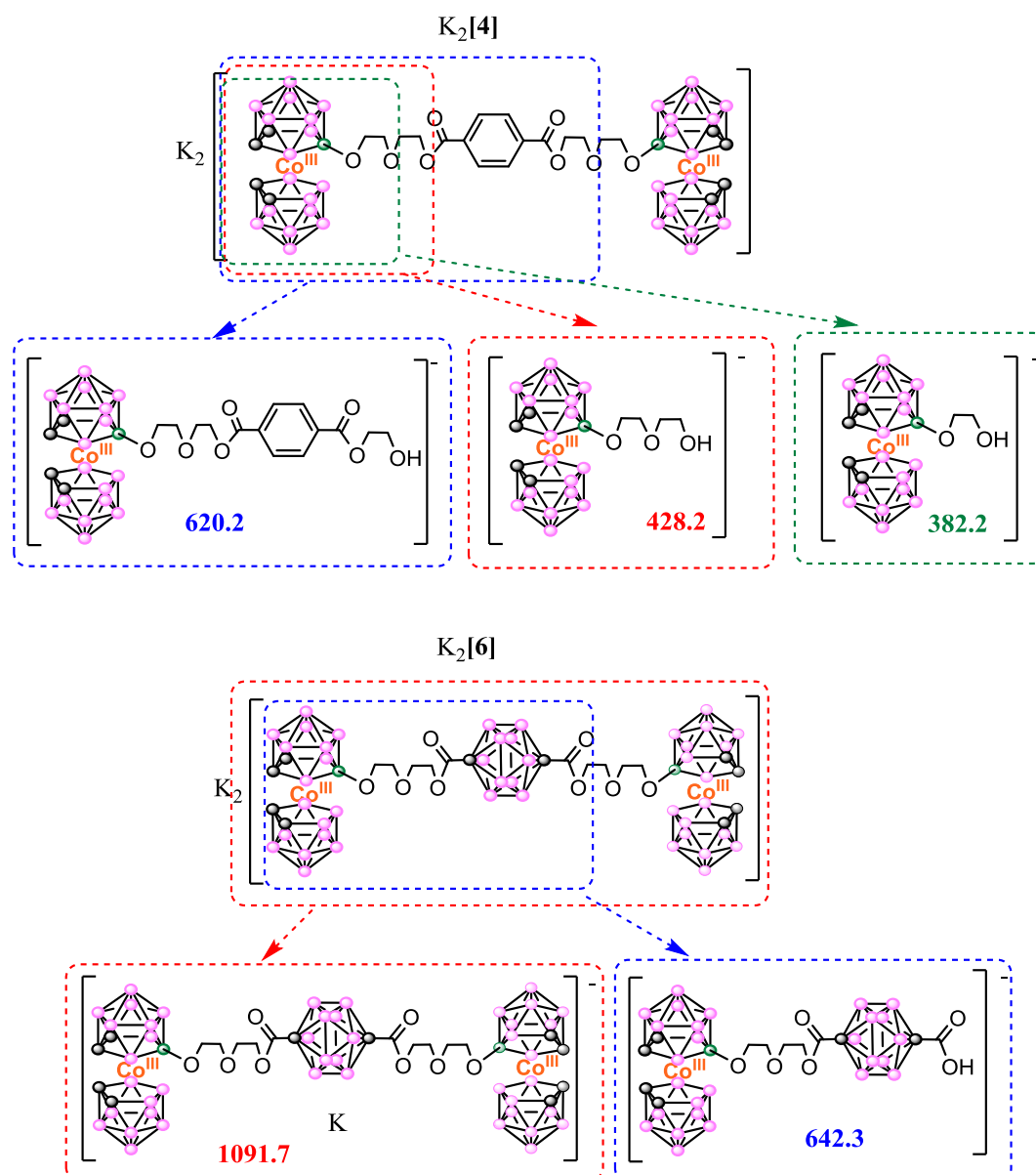


Figure 4. Fragmentation of the dianions $[4]^{2-}$ and $[6]^{2-}$ species into their monoanion fragments.

The ESI-MS spectrum of $K_2[6]$ displays three major peaks (Figure 5). The peak at 525.8 with a separation between isotopic peaks distribution of 0.50 m/z units corresponds to $M/2$ with M as the molecular weight of the dianion $[6]^{2-}$. The peak at 642.3 matches to the monoanionic fragment $[8-(HOOC-C_2B_{10}H_{10}-COO-(CH_2CH_2O)_2-3,3'-Co(1,2-C_2B_9H_{10})(1,2-C_2B_9H_{11})]^-$, while the third peak at 1091.7 agrees with the monoanion consisting of K^+ and $[6]^{2-}$. The peak at 1091.7 assigned as $[(M + K)]^-$ would be due to the strong $O \cdots K \cdots O$ interactions between the cation K^+ and the polyether chains

in the dianion and $K^+ \cdots H-B$ interactions between the cation and the hydrogen atoms of the B-H of the carborane. This behavior was observed in a similar anionic diethylene glycol cobaltabis(dicarbollide) species containing K^+ as cation.¹³ The peak of $[(M+K)]^-$ was not present in the dinuclear compound $K_2[4]$ which suggests structural differences exist between the two $K_2[4]$ and $K_2[6]$ salts due to the presence of the 2D phenylene and 3D carboranylene linkers respectively. Intramolecular interactions between K^+ and the accessible B-H vertices of the carboranyl group, which are hydridic, may be responsible for the observed mass spectra differences between the linkers in $K_2[4]$ and $K_2[6]$.

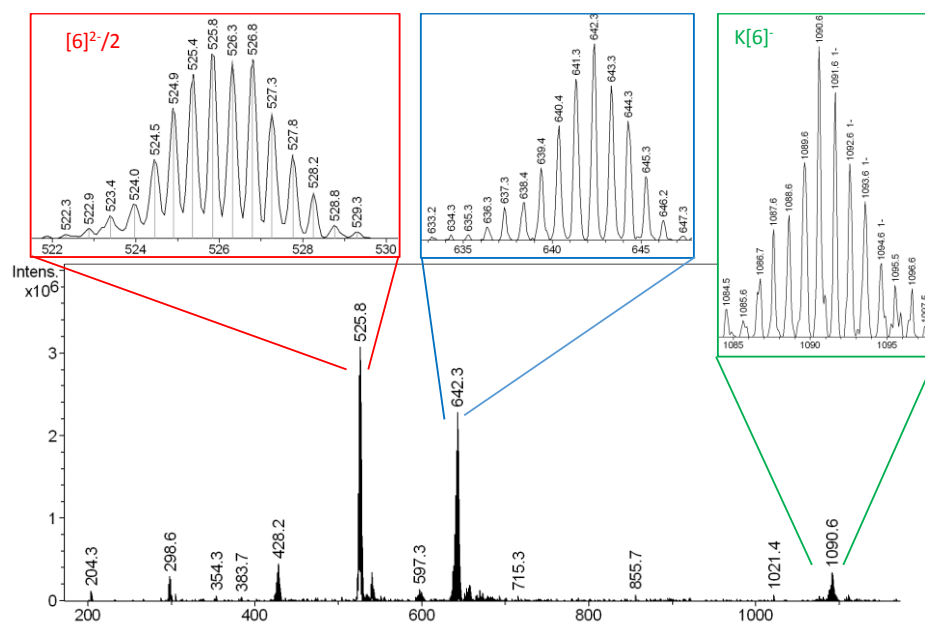
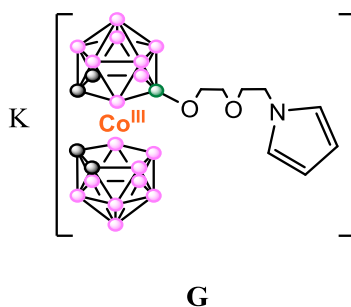


Figure 5. ESI-MS spectra of $K_2[6]$.

Thermogravimetric profiles were recorded for the new salts $Na[3]$, $K_2[4]$, $Na[5]$ and $K_2[6]$. The compound $Na[3]$ gave only one weight loss of 23.3 % at around 200 °C corresponding to the removal of the $C_2H_4OCO-1,4-C_6H_5$ group. Salt $Na[5]$ revealed three distinct weight losses with a total elimination of 32.32 % in the temperature range 150 – 550 °C in accord with the breaking of the $C_2H_4OCO-1,12-C_2B_{10}H_{11}$ branch. The dianionic compound $K_2[4]$ had two weight losses totaling 28.44 % in the temperature range of 150

– 470 °C and points to the elimination of the $(\text{C}_2\text{H}_4\text{OC}_2\text{H}_4\text{OCO})_{2-1,4}\text{-C}_6\text{H}_4$ fragment. However, the TGA of the dianionic $\text{K}_2[\mathbf{6}]$ complex displays only one weight loss of 35.5 % at around 150 °C which may correspond to the removal of the central $(\text{C}_2\text{H}_4\text{OC}_2\text{H}_4\text{OCO})_{2-1,12}\text{-C}_2\text{B}_{10}\text{H}_{10}$ part of the $[\mathbf{6}]^{2-}$ dianion.

Geometry optimizations by hybrid-DFT computations on the anions of $\text{Na}[\mathbf{3}]$, $\text{K}_2[\mathbf{4}]$, $\text{Na}[\mathbf{5}]$ and $\text{K}_2[\mathbf{6}]$ within the salts revealed the expected geometries of the discrete anions $[\mathbf{3}]^-$, $[\mathbf{4}]^{2-}$, $[\mathbf{5}]^-$ and $[\mathbf{6}]^{2-}$ (Figures S38-S41). As the salt $\text{K}_2[\mathbf{6}]$ revealed a $\text{K}[\mathbf{6}]^-$ peak in the ESI MS and clear K^+ interactions with the B-H hydrogens of the *para*-carborane unit in solution in the $^1\text{H}\{^{11}\text{B}\}$ NMR spectrum, the $\text{K}[\mathbf{6}]^-$ geometries were looked at computationally. In the most stable minimum of $\text{K}[\mathbf{6}]^-$ located, there are two close $\text{K}^+\dots\text{O}$ interactions, three $\text{K}^+\dots\text{H-B}$ interactions with the cobaltabis(dicarbollide) cluster and two $\text{K}^+\dots\text{H-B}$ interactions with the *para*-carborane cluster (Figure 6). The strong K^+ binding with the dianion $[\mathbf{6}]^{2-}$ observed experimentally is supported by hybrid-DFT computations. The environment at K^+ in $\{\text{K}[\mathbf{6}]\}^-$ is remarkably similar to the X-ray crystal structure of the potassium salt of the pyrrolyl cobaltabis(dicarbollide) **G** where two close $\text{K}^+\dots\text{O}$ interactions and three $\text{K}^+\dots\text{H-B}$ interactions with the cobaltabis(dicarbollide) cluster were present.¹³



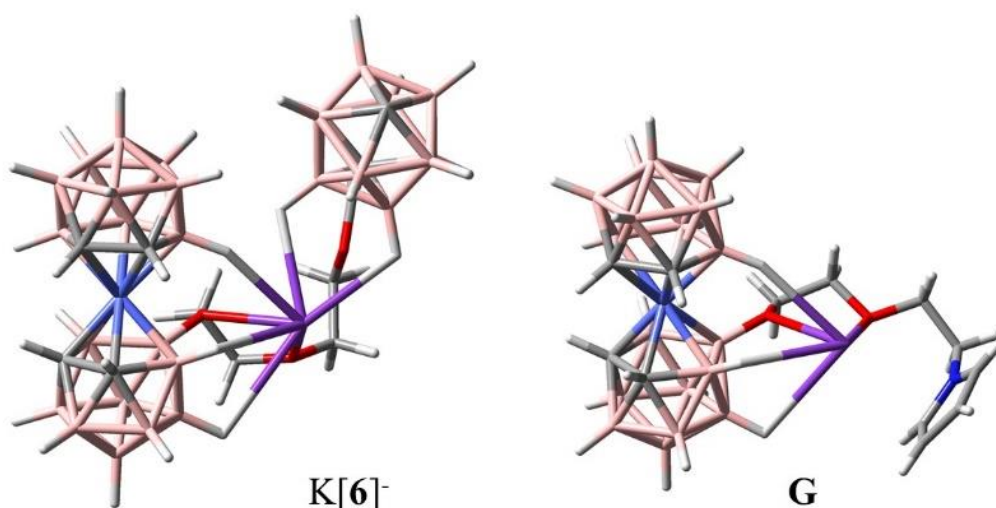


Figure 6. Comparison of the K^+ environments in the optimized geometry of $K[6]^-$ and the X-ray geometry of **G**. The second cobaltabis(dicarbollide)-diethylene glycol arm attached to the *para*-carborane in $K[6]^-$ is omitted for clarity.

Antimicrobial studies

The antimicrobial properties of the four new high boron content compounds $Na[3]$, $K_2[4]$, $Na[5]$ and $K_2[6]$ were tested along with the commercially available salt $Cs[1]$ using microdilution tests to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against clinical isolates; including 5 Gram-negative bacteria (3 *Escherichia coli* and 2 *Pseudomonas aeruginosa* strains), 4 Gram-positive bacteria (2 *Enterococcus faecalis* and 2 *Staphylococcus aureus* strains), and 3 yeast *Candida albicans* strains. These results, presented in Table 2, revealed that the five compounds mainly have good antibacterial activity against Gram-positive bacteria (MICs ranging from 1 to 16 mg/L), and moderate antifungal activity for *Candida albicans* (MICs ranging from 4 to 32 mg/L). None of the salts showed antibacterial effects against the clinical isolates of Gram-negative bacteria *E. coli* and *P. aeruginosa* (MICs ranged from 256 to >512 mg/L). MBC values indicated that these compounds have a bactericidal effect rather than bacteriostatic in most cases for Gram-

positive bacteria, since the ratio MBC/MIC was ≤ 4 , except for Na[3] against *E. faecalis* 190093 (MBC/MIC=32). In the case of *Candida albicans*, the MBC values of Na[5] compound against two of the isolates (*C. albicans* 180228 and *C. albicans* 181721) were higher than the MIC $\times 2$ what suggest this compound could have a fungistatic effect rather than fungicidal, whereas a fungicidal effect was confirmed for Na[3], K₂[4], K₂[6] and Cs[1]. Of note, no difference in MIC or MBC was found between the wild type *S. aureus* and the MRSA strains, which suggests that the mechanisms of the MRSA strain do not confer resistance to the present compounds.

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (indicated as mg/L) of cobaltabis(dicarbollides) on clinical isolates cause of infection. § Multidrug-resistant strains.

	Na[3]		K ₂ [4]		Na[5]		K ₂ [6]		Cs[1]	
Strain	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria										
<i>E. coli</i> LF82 §	>512	>512	>512	>512	>512	>512	>512	>512	256	512
<i>E. coli</i> WT 190940	>512	>512	>512	>512	>512	>512	>512	>512	256	256
<i>E. coli</i> BLEE 192348 §	>512	>512	>512	>512	>512	>512	>512	>512	256	256
<i>P. aeruginosa</i> WT 190089	>512	>512	512	>512	>512	>512	512	>512	256	256
<i>P. aeruginosa</i> IMI-R 187182 §	>512	>512	512	>512	>512	>512	512	>512	256	256
Gram-positive bacteria										
<i>E. faecalis</i> WT 190093	1	32	2	4	4	8	2	4	16	32
<i>E. faecalis</i> WT 194844	1	4	2	4	4	8	4	8	16	16
<i>S. aureus</i> WT 180895	1	4	8	16	16	32	4	16	16	32
<i>S. aureus</i> MRSA 182851 §	1	2	8	16	16	32	$\leq 4^*$	$\leq 16^*$	16	32
Yeast										
<i>C. albicans</i> 180228	16	16	4	4	32	>128	4	8	32	64
<i>C. albicans</i> 181721	16	32	4	$\leq 16^*$	32	>128	4	8	32	64
<i>C. albicans</i> 191026	16	16	4	4	32	32	4	8	32	64

*Consistent results could not be obtained. The value indicates the maximum MIC or MBC from four assays.

Since the antimicrobial properties for H[**1**]²³ and Na[**1**]²⁶ have been reported elsewhere, comparison between the reported MIC data for Na[**1**] and our MIC data for Cs[**1**] reveals similarities and differences. The microbial strains tested are not identical but salts of [1]⁻ clearly have antibacterial effects on Gram-positive bacteria and no antibacterial effects on Gram-negative bacteria. It seems that Na[**1**] is more effective than Cs[**1**] for Gram-positive bacteria with MIC₈₀ of *ca* 2.7 mg/L for Na[**1**] and MIC of *ca* 16 mg/L for Cs[**1**] respectively. An antimicrobial study on different [1]⁻ salts with identical microbial strains is desirable to confirm that different cations influence the antimicrobial properties of these salts. In concordance with our results, the reported anionic and zwitterion cobaltabis(dicarbollide) derivatives, **C-F** (Chart 2), selectively inhibited the growth of Gram-positive but not Gram-negative bacteria meaning a relatively narrow antimicrobial spectrum.²⁴⁻²⁶ Other anionic cobaltabis(dicarbollide) derivatives, **A** and **B** (Chart 2), have wide antimicrobial ranges as they were also effective against Gram-negative bacteria.²³

The cobaltabis(dicarbollide) derivatives, Na[**3**], K₂[**4**], Na[**5**] and K₂[**6**], examined showed a remarkable higher antibacterial effect on Gram-positive bacteria. For example, the MIC values for compounds **A-C** are 10, 13 and 8 mg/L respectively for the MRSA strain^{23,24} whereas the salt Na[**3**] showed the highest inhibitory effect to *S. aureus* with a MIC of 1 mg/L and an MBC of 2 mg/L towards MRSA strain 182851. This strain is resistant to penicillin, amoxicillin/clavulanic acid, oxacillin, tobramycin, amikacin, ciprofloxacin, levofloxacin and erythromycin (Table S1). The cobaltabis(dicarbollide) derivative **C** was reported to kill *S. aureus* MRSA by inducing an increase in the reactive oxygen species production,²⁴ what in turn induced irreversible damage to the cell wall/membrane. The antifungal activities against the studied *C. albicans* strains were

similar to compounds **A** and **B** described by some of us earlier.²³ The larger assemblies [K]₂[**4**] and K₂[**6**] displayed the highest fungal potencies with MICs of 4 mg/L.

All the new anionic [**3**]⁻-[**6**]²⁻ derivatives possess a hydrophilic-lipophilic balance: hydrophilic from the anionic ethylene glycol cobaltabis(dicarbollide) clusters and lipophilic from the aryl or *p*-carboranyl groups. From a consideration of the structure-activity relationships, it seems reasonable to conclude that the introduction of two hydrophilic ethylene glycol cobaltabis(dicarbollide) anions bonded to the lipophilic phenyl or *p*-carboranyl groups leads to the increased antifungal activity. The 3D *p*-carborane unit, which is more lipophilic than the aromatic unit, provide the highest antifungal activity to [**6**]²⁻. Moreover, the higher electron-withdrawing character of the *closo* carborane at the carbon atom compared to the aromatic ring may be the difference in biological activity between [**6**]²⁻ and [**4**]²⁻.

The fact that these compounds are selective for Gram-positive bacteria and fungi not only questions the mechanism of action but also suggests that the outer membrane and/or the expression of efflux pumps of Gram-negative bacteria may protect from the action of these molecules. Future studies are needed to demonstrate the mechanism of action of these compounds. Moreover, regarding their antimicrobial potential, new cobaltabis(dicarbollide) variants like **A** and **B** with increased effectiveness against Gram-negative bacteria are of great interest since Gram-negative multi-resistant bacteria are on the top of the global priority list for research and development of new drugs (WHO).

Conclusions

New hybrid assemblies containing cobaltabis(dicarbollide) anions and aromatic or carborane units with enhanced water solubility were synthesized to explore their potential as suitable antimicrobial agents. The electronic, hydrophobic, and steric

differences between 2D aromatic ring and 3D aromatic carborane cluster provide different hydrophilic-lipophilic balances in the new compounds and result in differences in their antibacterial and antifungal activities. These cobaltabis(dicarbollide) assemblies tested here are potential narrow-spectrum antimicrobial agents, with very good bactericidal effect (MIC 1-8 mg/L) against Gram-positive bacteria and antifungal effect (MIC 4 mg/L) in the case of the larger assemblies ($K_2[4]$ and $K_2[6]$) against *C. albicans*. In particular, one compound ($Na[3]$) is very promising as an antimicrobial agent because low doses (MIC 1 mg/L) destroy the methicillin-resistant *S. aureus* (MRSA) strain we have evaluated, and this organism is classified as a high priority pathogen for R&D of new antibiotics by the World Health Organization. The ineffectiveness of these assemblies against Gram-negative bacteria suggests that decreased intracellular accumulation may hamper the antibacterial action of these compounds, making Gram-negative bacteria naturally protected. Studying the mechanism of action in these compounds may shed light on the design of new cobaltabis(dicarbollide) assemblies with effective antimicrobial activities on Gram-negative pathogens.

Experimental section

Chemicals

Materials and instrumentation

All reagents in the present work were obtained from Sigma-Aldrich and used without further purification. Reagent grade organic solvents were obtained from Carlo Erba and high purity de-ionized water was obtained by passing distilled water through a nano-pure Milli-Q water purification system.

All manipulations were carried out under nitrogen atmosphere. Dry DME was distilled from sodium benzophenone prior to use. All complexes and ligands 1-COOH-

1,12-C₂B₁₀H₁₁ and 1,12-(COOH)₂-1,12-C₂B₁₀H₁₀,²⁸ [Cs][3,3'-Co(1,2-C₂B₉H₁₁)₂][Cs][1],²⁹ [3,3'-Co(8-C₄H₈O₂-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)] **2**,^{11,12} and [N(CH₃)₄][3,3'-Co(8-O(CH₂CH₂O)₂C(O)C₆H₅-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)]³⁰ were prepared according to the literature.

IR spectra (ν , cm⁻¹) were obtained using the Shimadzu FTIR-8300 spectrophotometer. UV-Vis spectroscopy was performed on a Cary 50 Scan (Varian) UV-Vis spectrophotometer with 1 cm quartz cells. The ¹H and ¹H{¹¹B}-NMR (300.13/400.13 MHz), ¹³C{¹H}-NMR (75.47 MHz) and ¹¹B and ¹¹B{¹H}-NMR (96.29 /128.37 MHz) spectra were recorded on a Bruker ARX 300 or a DPX 400 instrument equipped with the appropriate decoupling accessories. All NMR spectra were performed in the indicated deuterated solvent at 22°C. ¹¹B and ¹¹B{¹H}-NMR chemical shifts were referenced to external BF₃·OEt₂ at 0.0 ppm while the ¹H, ¹H{¹¹B}, and ¹³C{¹H}-NMR shifts were referenced to SiMe₄ at 0.0 ppm. Elemental analyses were performed using a CHNS-O Elemental Analyzer EA-1108 from Fisons. ESI-MS experiments were recorded on a Navigator LC/MS chromatography from Thermo Quest Finnigan, using acetonitrile as a mobile phase. Thermogravimetric analysis (TGA) was performed using a Netzsch STA 449 thermal analyzer at a heating rate of 10 °C min⁻¹ under an Ar atmosphere.

Synthesis of Na[1-COO-1,12-C₂B₁₀H₁₁].

This ligand was prepared by neutralization of the 1-COOH-1,12-C₂B₁₀H₁₁ acid. Dried sodium carbonate (17 mg, 0.16 mmol) and 1-COOH-1,12-C₂B₁₀H₁₁ (60 mg, 0.319 mmol) were dissolved in 15 mL of distilled water at room temperature. After stirring 2 hours, the solvent was removed. The resulting solid was filtered off and dried in vacuum. Yield: 65 mg (97 %) of pure Na[1-COO-1,12-C₂B₁₀H₁₁] was obtained. IR ν (cm⁻¹): 3306 (O-Na), 3053 (C_c-H), 2888 (C-H)_{alkyl}, 2600 (B-H), 1626 (C=O), 1347 δ (CH). ¹H NMR (400 MHz,

((CD₃)₂CO): δ 3.35 (s, 1H, C-H), 3.10-1.4 (m, 10H, B-H). ¹H{¹¹B} NMR (400 MHz, ((CD₃)₂CO): δ 3.35 (s, 1H, C-H), 2.40 (s, 5H, B-H), 2.13 (s, 5H, B-H). ¹¹B NMR (96.3 MHz, ((CD₃)₂CO): δ -10.0 (d, ¹J(B,H) = 176 Hz, 5B), -12.5 (d, ¹J(B,H) = 176 Hz, 5B).

Synthesis of Na[3,3'-Co(8-O(CH₂CH₂O)₂C(O)C₆H₅-1,2-C₂B₁₀H₁₀)(1',2'-C₂B₉H₁₁)], Na[3].

A yellowish ion exchange resin was kept in HCl solution (3 M) for 24 h then filled into a column. A solution of HCl (3M) was passed through this column until a transparent solution appeared from the column. Excess HCl was removed by washing the resin using distilled water till the exit solution had neutral pH. A solution of NaCl (3M) was then passed slowly through the column to change H⁺ ions into Na⁺ ions on the column. Distilled water was used to remove excess NaCl. An acetonitrile/water (50/50) mixture was passed through the column and a minimum amount of the same solution was used to dissolve a sample of complex [N(CH₃)₄][3]. The orange solution was passed through the column several times then the solvent was evaporated from the orange solution *in vacuo* to give 90% yield of the Na[3] compound. FTIR-ATR: ν = 3038 (C_c-H), 2918, 2865 (C-H)_{alkyl}, 2528 (B-H), 1699 (C=O), 1440 δ (CH₂), 1279 δ (CH), 1131 (C-O-C). ¹H NMR ((CD₃)₂CO): δ = 8.06 (m, 2H, C₆H₅), 7.64 (t, 1H, C₆H₅), 7.53 (t, 2H, C₆H₅), 4.30 (t, 2H, OCH₂CH₂), 4.30 (br s, 4H, C_c-H), 3.84 (t, 2H, OCH₂CH₂), 3.69 (m, 4H, OCH₂CH₂). ¹H{¹¹B} NMR ((CD₃)₂CO): δ = 8.06 (m, 2H, C₆H₅), 7.64 (t, 1H, C₆H₅), 7.53 (t, 2H, C₆H₅), 4.30 (t, 2H, OCH₂CH₂), 4.30 (br s, 4H, C_c-H), 3.84 (t, 2H, OCH₂CH₂), 3.69 (m, 4H, OCH₂CH₂), 2.79, 2.71, 2.45, 2.01, 1.80, 1.67, 1.58, 1.47 (B-H). ¹³C{¹H} NMR ((CD₃)₂CO): δ = 165.87 (s, COO), 132.88 (s, C₆H₅), 129.40 (s, C₆H₅), 128.46 (s, C₆H₅), 71.03 (s, OCH₂), 68.81 (s, OCH₂), 68.50 (s, OCH₂), 64.31 (s, OCH₂), 54.36 (s, C_c-H). ¹¹B NMR ((CD₃)₂CO): δ = 23.0 (s, 1B, B(8)), 4.0 (d, ¹J(B,H) = 151 Hz, 1B), 0.6 (d, ¹J(B,H)

= 141 Hz, 1B), -2.4 (d, $^1J(\text{B},\text{H}) = 154$ Hz, 1B), -3.4 (d, $^1J(\text{B},\text{H}) = 146$ Hz, 2B), -7.5 (d, $^1J(\text{B},\text{H}) = 102$ Hz), -8.3 (d, $^1J(\text{B},\text{H}) = 94$ Hz, 4B), -17.1 (d, $^1J(\text{B},\text{H}) = 156$ Hz, 2B), -20.3 (d, $^1J(\text{B},\text{H}) = 156$ Hz, 2B,), -21.8 (d, $^1J(\text{B},\text{H}) = 147$ Hz, 1B), -28.2 (d, $^1J(\text{B},\text{H}) = 170$ Hz, 1B). ESI-MS (m/z): 532.2 (M, 100%) where M is the molecular weight of the anion, $[\text{3,3'}\text{-Co(8-O(CH}_2\text{CH}_2\text{O)}_2\text{C(O)C}_6\text{H}_5\text{-1,2-C}_2\text{B}_9\text{H}_{10})(\text{1',2'-C}_2\text{B}_9\text{H}_{11})]^-$, **[3]**⁻.

Synthesis of $\text{K}_2[\text{1'',4''-}\{3,3'\text{-Co(8-O(CH}_2\text{CH}_2\text{O)}_2\text{CO-1,2-C}_2\text{B}_9\text{H}_{10})(\text{1',2'-C}_2\text{B}_9\text{H}_{11})\}_2\text{-C}_6\text{H}_4]$, $\text{K}_2[\text{4}]$.

Under an inert atmosphere, a mixture of dried terephthalic acid (10.1 mg, 0.061 mmol) and potassium carbonate (67.4 mg, 0.487 mmol) were refluxed in 10 mL of DME for 2 h. Then, a solution of **2** (50 mg, 0.122 mmol) in 5 mL of DME was added dropwise to the solution. After refluxing overnight, the excess of potassium carbonate was filtered off and discarded. The solvent was removed and washed with petroleum ether. The obtained compound was dissolved in water and acetonitrile, then filtrated. The solvent was removed. The obtained orange powder was passed twice through a column chromatography on silica ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1) to give a yellow-orange fraction. The obtained solid was washed twice with 10 mL of diethyl ether and dried in vacuum to give 34.8 mg (53.7 %) of $\text{K}_2[\text{4}]$. Elemental analysis for $\text{C}_{24}\text{H}_{62}\text{B}_{36}\text{Co}_2\text{O}_8\text{K}_2$: Calc.: C, 27.09; H, 5.84. Found: C, 26.96; H, 5.65. FTIR-ATR: $\nu = 3021$ ($\text{C}_c\text{-H}$), 2969, 2900 ($\text{C-H}_{\text{alkyl}}$), 2533 (B-H), 1713 (C=O), 1446, 1407 $\delta(\text{CH}_2)$, 1254 $\delta(\text{CH})$, 1078, 1020 (C-O-C). ^1H NMR ($(\text{CD}_3)_2\text{CO}$): $\delta = 8.16$ (s, 4H, C_6H_4), 4.47 (t, 4H, OCH_2CH_2), 4.26 (br s, 8H, $\text{C}_c\text{-H}$), 3.85 (t, 4H, OCH_2CH_2), 3.60 (m, 8H, OCH_2CH_2). $^{13}\text{C}\{^1\text{H}\}$ NMR ($(\text{CD}_3)_2\text{CO}$): δ 166.09 (s, COO), 134.96 (s, C_6H_4), 130.37 (s, C_6H_4), 72.71 (s, OCH_2), 71.76 (s, OCH_2), 69.51 (s, OCH_2), 65.50 (s, OCH_2), 55.19 (s, $\text{C}_c\text{-H}$), 47.22 (s, $\text{C}_c\text{-H}$). ^{11}B NMR ($(\text{CD}_3)_2\text{CO}$): δ 23.0 (s, 2B, B(8)), 4.0 (s, 2B), 0.4 (s, 2B), -2.5 (s, 2B), -4.4 (s, 4B), -7.4 (s, 4B), -8.3 (s, 8B), -17.4 (s, 4B), -20.5 (s, 4B), -22.2 (s, 2B), -28.8 (s, 2B). ESI-MS (m/z): 382,2 ($[\text{3,3'}\text{-Co(8-}$

OCH₂CH₂O-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)⁻, 60%); 428.2 [3,3'-Co (8-(CH₂CH₂O)₂OH-1,2-C₂B₉H₁₀)(1,2-C₂B₉H₁₁)⁻, 60%); 493.2 (M/2, 100%); 506.7 ((M+C₂H₃)/2, 45%), where M is the molecular weight of the dianion, [1'',4''-{3,3'-Co(8-O(CH₂CH₂O)₂CO-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)}₂-C₆H₄]²⁻, [4]²⁻, and 620.2 ([3,3'-Co(8-HOCH₂CH₂-OOC-C₆H₄-COO-(CH₂CH₂O)₂-1,2-C₂B₉H₁₀)(1,2-C₂B₉H₁₁)⁻, 11%).

Synthesis of Na[3,3'-Co(8-O(CH₂CH₂O)₂C(O)C₂B₁₀H₁₁-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)], Na[5].

Under an inert atmosphere, dried Na[1-COO-1,12-C₂B₁₀H₁₁] (26 mg, 0.122 mmol) and **2** (50 mg, 0.122 mmol) were refluxed in 10 mL of anhydrous DME. After stirring over-weekend, the solvent was removed. This was filtered off, washed with petroleum ether and dried in vacuum; 49.3 mg (60%) of Na[5] was obtained. Elemental Analysis for C₁₁H₄₀B₂₈CoO₄Na: Calc: C, 21.27; H, 6.49. Found: C, 21.13; H, 6.22. FTIR-ATR: ν= 3400,1706 (H₂O), 3048 (C_c-H), 2914, 2871, 2826 (C-H)_{alkyl}, 2534 (B-H), 1714 (C=O), 1456 δ(CH₂), 1188, 1121 (C-O-C). ¹H NMR ((CD₃)₂CO): δ= 4.27 (br s, 4H, C_c-H), 4.16 (t, 2H, OCH₂CH₂), 3.66 (t, 2H, OCH₂CH₂), 3.58 (t, 2H, OCH₂CH₂), 3.52 (t, 2H, OCH₂CH₂), 3.32 (s, 1H, *para*-carborane C_c-H). ¹H{¹¹B} NMR ((CD₃)₂CO): δ= 4.28 (br s, 4H, C_c-H), 4.17 (t, 2H, OCH₂CH₂), 4.00 (s, 1H, C_c-H), 3.65 (t, 2H, OCH₂CH₂), 3.58 (t, 2H, OCH₂CH₂), 3.52 (t, 2H, OCH₂CH₂), 3.32 (s, 1H, *para*-carborane C_c-H), 2.93 (s, 4H, B-H), 2.78 (s, 2H, B-H), 2.71 (s, 1H, B-H), 2.43 (s, 6H, B-H + *para*-carborane B-H), 2.22 (s, 5H, *para*-carborane B-H), 2.01 (s, 2H, B-H), 1.80 (s, 2H, B-H), 1.67 (s, 2H, B-H), 1.58 (s, 2H, B-H), 1.47 (s, 1H, B-H). ¹³C{¹H} NMR ((CD₃)₂CO): δ= 162.36 (s, COO), 72.78 (s, C-COO), 70.65 (s, OCH₂), 69.41 (s, OCH₂), 69.05 (s, OCH₂), 67.63 (s, OCH₂), 55.31 (s, C_c-H), 47.30 (s, C_c-H), 41.32 (s, C-H). ¹¹B NMR ((CD₃)₂CO): δ= 23.0 (s, 1B, B(8)), 3.9 (d, ¹J(B,H) = 136 Hz, 1B), 0.4 (d, ¹J(B,H) = 142 Hz, 1B), -2.6 (d, ¹J(B,H) = 157

Hz, 1B), -4.3 (d, $^1J(\text{B,H}) = 162$ Hz, 4B), -7.3 (d, $^1J(\text{B,H}) = 97$ Hz, 2B), -8.6 (d, $^1J(\text{B,H}) = 100$ Hz, 4B), -13.5 (d, $^1J(\text{B,H}) = 171$ Hz, 5B), -15.3 (d, $^1J(\text{B,H}) = 175$ Hz, 5B), -19.0 (d, $^1J(\text{B,H}) = 152$ Hz, 2B), -20.6 (d, $^1J(\text{B,H}) = 157$ Hz, 2B), -22.1 (d, $^1J(\text{B,H}) = 140$ Hz, 2B), -28.7 (d, $^1J(\text{B,H}) = 154$ Hz, 2B). ESI-MS (m/z): 598.4 (M, 100%) where M is the molecular weight of the anion, $[3,3'\text{-Co(8-O(CH}_2\text{CH}_2\text{O)}_2\text{C(O)-1,2-C}_2\text{B}_9\text{H}_{10})(1',2'\text{-C}_2\text{B}_9\text{H}_{11})\text{-1,12-C}_2\text{B}_{10}\text{H}_{11}]^-$, [5]⁻.

Synthesis of $\text{K}_2[1'',12''\text{-}\{3,3'\text{-Co(8-O(CH}_2\text{CH}_2\text{O)}_2\text{CO-1,2-C}_2\text{B}_9\text{H}_{10})(1',2'\text{-C}_2\text{B}_9\text{H}_{11})\}_2\text{-C}_2\text{B}_{10}\text{H}_{10}]$, $\text{K}_2[6]$.

Under an inert atmosphere, a mixture of dried 1,12-(COOH)₂-C₂B₁₀H₁₀ (23 mg, 0.1 mmol) and potassium carbonate (107 mg, 0.775 mmol) were refluxed in 10 mL of anhydrous acetone for 2 hours. Then, a solution of **2** (80 mg, 0.195 mmol) in 5 mL of anhydrous acetone was added dropwise in the solution. After stirring overnight, the excess of potassium carbonate was filtered off and discarded. The solvent was removed, washed with petroleum ether and dried in vacuum. Yield: 103 mg (94%) was obtained. Elemental Analysis for C₂₀H₆₈B₄₆Co₂O₈K₂: Calc: C, 21.26; H, 6.06. Found: C, 21.11; H, 6.27. FTIR-ATR: $\nu = 3039$ (C_c-H), 2961, 2921, 2881 (C-H)_{alkyl}, 2532 (B-H), 1702 (C=O), 1440, 1411 $\delta(\text{CH}_2)$, 1257 $\delta(\text{CH})$, 1105, 1020 (C-O-C). $^1\text{H NMR}$ ((CD₃)₂CO): $\delta = 4.25$ (br s, 8H, C_c-H), 4.16 (m, 4H, OCH₂CH₂), 3.63 (m, 4H, OCH₂CH₂), 3.57 (m, 4H, OCH₂CH₂), 3.50 (m, 4H, OCH₂CH₂). $^1\text{H}\{^{11}\text{B}\}$ NMR ((CD₃)₂CO): $\delta = 4.25$ (br s, 8H, C_c-H), 4.16 (m, 4H, OCH₂CH₂), 3.63 (m, 4H, OCH₂CH₂), 3.57 (m, 4H, OCH₂CH₂), 3.50 (m, 4H, OCH₂CH₂), 2.92 (s, 8H, B-H), 2.75 (s, 6H, B-H), 2.49 (s, 4H, B-H + *para*-carborane B-H), 2.44 (s, 4H, *para*-carborane B-H), 2.40 (s, 4H, *para*-carborane B-H), 2.01 (s, 4H, B-H), 1.81 (s, 4H, B-H), 1.67 (s, 4H, B-H), 1.58 (s, 4H, B-H), 1.49 (s, 2H, B-H). $^{13}\text{C}\{^1\text{H}\}$ NMR ((CD₃)₂CO): $\delta = 163.81$ (s, COO), 72.86 (s, C-COO), 69.48 (s, OCH₂), 69.10 (s,

OCH₂), 67.81 (s, OCH₂), 67.65 (s, OCH₂), 55.25 (s, C_c-H), 47.35 (s, C_c-H). ¹¹B NMR ((CD₃)₂CO): δ= 24.2 (s, 2B, B(8)), 5.2 (d, ¹J(B,H)= 135Hz, 2B), 1.6 (d, ¹J(B,H)= 143 Hz, 2B), -1.4 (d, ¹J(B,H)= 153 Hz, 2B), -3.0 (d, ¹J(B,H)= 167 Hz, 2B), -6.0 (d, ¹J(B,H)= 104 Hz, 4B), -7.2 (d, ¹J(B,H)= 114 Hz, 6B), -12.8 (d, ¹J(B,H) = 169 Hz, 10B), -16.1 (d, ¹J(B,H) = 159 Hz, 4B), -19.2 (d, ¹J(B,H) = 153 Hz, 4B), -20.9 (d, ¹J(B,H) = 163 Hz, 2B), -27.3 (d, ¹J(B,H) = 140 Hz, 2B). ESI-MS (m/z): 525.8 (M/2, 100%), 642.3 (3,3'-Co(8-(HOOC-C₂B₁₀H₁₀-COO-(CH₂CH₂O)₂-1,2-C₂B₉H₁₀)(1,2-C₂B₉H₁₁)]⁻, 73%), 1091.7 (M + K⁺, 57%); where M is the molecular weight of the dianion, [1'',12''-{3,3'-Co(8-O(CH₂CH₂O)₂CO-1,2-C₂B₉H₁₀)(1',2'-C₂B₉H₁₁)}₂-1'',12''-C₂B₁₀H₁₀]²⁻, [6]²⁻.

Microorganisms and growth media.

Clinical bacterial (N=8) and yeast (N=3) isolates responsible for human infections were used to test the antimicrobial properties of the compounds. These microorganisms were isolated at the Hospital Reina Sofía of Córdoba (Spain) in 2018 and 2019. In particular, as Gram-negative bacteria, two strains of *Escherichia coli* and two *Pseudomonas aeruginosa* were tested, including strains susceptible to commonly used antimicrobials (*E. coli* 190940 and *P. aeruginosa* 190089) or multi-drug resistant (*E. coli* BLEE 192348 and *P. aeruginosa* IMI 187182). *E. coli* strain LF82, previously isolated from a patient with ileal Crohn's disease was also included.³¹ As Gram-positive bacteria, we tested two *Enterococcus faecalis* (*E. faecalis* 190093 and *E. faecalis* 194844), and two *Staphylococcus aureus* (*S. aureus* MRSA 182851, which is multidrug-resistant, and *S. aureus* 180895). Yeast strains belonged to the species *Candida albicans* (*C. albicans* 180228, *C. albicans* 181721 and *C. albicans* 191026). Bacterial strains were phenotypically characterized by the Microscan Walkaway-96 System; the antimicrobial resistance profiles are presented in Table S1.

Luria-Bertani (LB) broth was used for growing the *E. coli* species, brain heart infusion (BHI) for *P. aeruginosa*, *E. faecalis* and *S. aureus*, and brain heart agar (BHA) for *C. albicans*.

Antimicrobial tests

Minimum inhibitory concentration (MIC) was determined by broth microdilution following standardized guidelines (International Standards Organisation, 2006), and the minimum bactericidal concentration (MBC) by counting viable cells at MIC and MIC×2 concentrations following the CLSI standard protocols (CLSI, 1999).

Bacterial suspensions

Overnight bacterial cultures were prepared in 1.5 mL of LB (*E. coli*) or BHI (*P. aeruginosa*, *E. faecalis* and *S. aureus*) in glass tubes with cotton plugs and incubating them for 16-18 h at 37 °C without shaking. Bacterial density was measured at an optical density of 620 nm and bacterial suspensions were prepared at a final concentration of 1×10^6 CFU / mL in cation-adjusted Mueller Hinton (MH) broth.

Yeast suspensions

Candida inoculums were prepared by resuspending 5 individual colonies in at least 3 mL of sterile water. Optical density was measured 530 nm and suspensions were prepared in sterile water at a final concentration of $1 - 5 \times 10^5$ CFU / mL.

Compounds stock solutions

The stock solutions were prepared at 1024 mg/l with sterile Milli Q water and kept at -20°C or at 4°C a maximum of 1 month. Na[5] and K₂[6] were first diluted with dimethyl sulfoxide (DMSO) in a concentration of 50 mg/ml and then sterile water was added until the final concentration was of 1024 mg/L. All the other compounds were diluted directly with water at 1024 mg/L.

MIC and MBC determinations

For bacterial tests, serial dilutions of the compounds were prepared in cation-adjusted Mueller Hinton broth in a polystyrene 96-well plate. Concentrations tested were 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0 mg/L. Bacteria were inoculated at a final concentration of 5×10^5 CFU/mL in final volume of 100 μ L. Microplates were incubated for 18 h at 37 °C in aerobic conditions.

The medium used to test *Candida* was RPMI with 2% glucose and L-glutamine, and the concentrations of the compounds tested were 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0 mg/L. The test was done in a final volume of 200 μ L with $1-5 \times 10^5$ CFU/mL per well. Microplates were incubated for 24 h at 37 °C in aerobic conditions. All tests were done in triplicate.

The MIC was determined as the lowest concentration of the compound (mg/L) needed to inhibit the growth of the microorganism, which was observed by the absence of visible growth in the well. The MBC was determined by plating 20 μ L of the suspension corresponding to the MIC and the subsequent one (MIC \times 2) into LB agar plates that did not contain the compound. The MBC was determined as the lowest concentration of compound that reduces the viability of the initial bacterial inoculum at $\geq 99.9\%$.

ASSOCIATED CONTENT

Supporting Information

Spectra (IR, ^1H , $^1\text{H}\{^{11}\text{B}\}$, $^{13}\text{C}\{^1\text{H}\}$, ^{11}B , $^{11}\text{B}\{^1\text{H}\}$, ESI-MS, TGA/DSC) and antibiotic resistant profiles (MIC; mg/L) against several Gram-positive bacteria. Cartesian coordinates for [1] $^-$, 2, [3] $^-$, [4] $^{2-}$, [5] $^-$, [6] $^{2-}$ and K[6] $^-$ optimized geometries.

Acknowledgments

This research was financed by MINECO (CTQ2016-75150-R, CTQ2015-66143-P, and SAF2017-82261-P, with the last grant cofunded by the European Regional Development Fund) and Generalitat de Catalunya (2017 SGR 1720).

Conflicts of interest

“There are no conflicts to declare”

References

-
- (1) Crowfoot Hodgkin, D.; Kamper, J.; Mackay, M.; Pickworth, J.; Trueblood, K.N.; White, J G. Dorothy Crowfoot Hodgkin was awarded the 1964 Nobel Prize for her determinations by X-ray techniques of the structures of important biochemical substances. *Nature*, **1956**, *178*, 64-66
- (2) (a) Rosenberg, B.; Van Kamp, L.; Krigas, T. Inhibition of cell division in escherichia coli by electrolysis products from a platinum electrode. *Nature*, **1965**, *178*, 698–699. (b) Kaim, W.; Schwederski, B.; Klein A. in Bioinorganic Chemistry - Inorganic Elements in the Chemistry of Life: An Introduction and Guide, 2nd Edition, 2013, John Wiley & Sons, Ltd, Chichester, U.K.
- (3) (a) Köpf-Maier, P.; Wagner, W.; Köpf, H. In vitro cell growth inhibition by metallocene dichlorides. *Cancer Chemother. Pharmacol.* **1981**, *5*, 237-241. (b) Köpf-Maier, P. Complexes of metals other than platinum as antitumour agents. *Eur. J. Clin. Pharmacol.* **1994**, *47*, 1-16.
- (4) Grimes R. N. “Carboranes” 3rd Ed. Elsevier Inc. New York, **2016**.
- (5) Olid D.; Nuñez R.; Viñas C.; Teixidor F. Methods to produce B–C, B–P, B–N and B–S bonds in boron clusters. *Chem. Soc. Rev.* **2013**, *42*, 3318-3336.
- (6) (a) Sivaev, I. B.; Bregadze, V. I. Chemistry of Cobalt bis(dicarbollides). A review. *Collect. Czech. Chem. Commun.* **1999**, *64*, 783. (b) Dash, B. P.; Satapathy, R.; Swain, B. R.; Mahanta C. S.; Jena, B. B.; Hosmane, N. S. Cobalt bis(dicarbollide) anion and its derivatives. *J. Organomet. Chem.* **2017**, *849-850*, 170-194.
- (7) Masalles, C.; Borrós, S.; Viñas, C.; Teixidor, F. Extraordinary Overoxidation resistance increase in self- doped polypyrroles by using non- conventional low charge- density anions. *Adv. Mater.* **2002**, *14*, 826-829.
- (8) (a) Hardie, M. J.; Raston, C. L. Solid state supramolecular assemblies of charged supermolecules (Na[2.2.2]cryptate)⁺ and anionic carboranes with host cyclotrimeratrylene. *Chem. Commun.* **2001**, *26*, 905-906. (b) Fox, M. A.; Hughes, A. K. Cage C-H...X interactions in solid-state structures of icosahedral carboranes. *Coord. Chem. Rev.* **2004**, *248(5-6)*, 457-476. (c) Brusselle, D.; Bauduin, P.; Girard, L.; Zaulet, A.; Viñas, C.; Teixidor, F.; Ly I.; Diat, O. Lyotropic lamellar phase

-
- formed from monolayered θ - shaped carborane- cage amphiphiles. *Angew. Chem. Int. Ed.* **2013**, 52 (46), 12114-12118.
- (9) Tarrés, M.; Viñas, C.; González-Cardoso, P.; Hänninen, M. M.; Sillanpää, R.; Āordovič, V.; Uchman, M.; Teixidor, F.; Matějček, P. Aqueous self- assembly and cation selectivity of cobaltabisdicarbollide dianionic dumbbells. *Chem. Eur. J.* **2014**, 20, 6786-6794.
- (10) (a) Bauduin, P.; Prevost, S.; Farras, P.; Teixidor, F.; Diat, O.; Zemb, T. A theta-shaped amphiphilic cobaltabisdicarbollide anion: transition from monolayer vesicles to micelles. *Angew. Chem. Int. Ed.*, **2011**, 50 (23), 5298-5300. (b) Viñas, C.; Tarres, M.; Gonzalez-Cardoso, P.; Farras, P.; Bauduin, P.; Teixidor, F. Surfactant behaviour of metallacarboranes. A study based on the electrolysis of water. *Dalton Trans.* **2014**, 13, 5062-5068. (c) Āordovič, V.; Tosner, Z.; Uchman, M.; Zhigunov, A.; Reza, M.; Ruokolainen, J.; Pramanik, G.; Cígler, P.; Kalíková, K.; Gradzielski, M.; Matějček, P. Stealth amphiphiles: self-assembly of polyhedral boron clusters. *Langmuir* **2016**, 32(26), 6713-6722. (d) Uchman, M.; Āordovič, V.; Tošner, Z.; Matějček, P. Classical amphiphilic behavior of nonclassical amphiphiles: a comparison of metallacarborane self- assembly with SDS micellization. *Angew. Chem. Int. Ed.*, **2015**, 54 (47), 14113-14117.
- (11) (a) Plešek, J.; Hermánek, S.; Franken, A.; Cisarova I.; Nachtigal C. Dimethyl sulfate induced nucleophilic substitution of the [bis(1,2-dicarbollido)-3-cobalt(1-)]ate ion. Syntheses, properties and structures of its 8,8'- μ -sulfato, 8-phenyl and 8-dioxane derivatives. *Collect. Czech. Chem. Commun.* **1997**, 62, 47-56. (b) Selucky, P.; Plešek, J.; Rais, J.; Kyrs M.; Kadlecova, L. Extraction of fission products into nitrobenzene with dicobalt tris-dicarbollide and ethyleneoxy-substituted cobalt bis-dicarbollide. *J. Radioanal. Nucl. Chem.* **1991**, 149, 131-140. (c) Plešek, J.; Stibr B.; Hermanek, S. A [8,8'- μ -I-3-Co(1,2-C₂B₉H₁₀)₂] metallacarborane complex with a iodonium bridge. Evidence for a bromonium analogue. *Collect. Czech. Chem. Commun.* **1984**, 49, 1492-1496.

-
- (12) Teixidor F.; Pedrajas J.; Rojo I.; Viñas C.; Kivekäs R.; Sillanpää R.; Sivaev I.; Bregadze V.; Sjöberg, S. Chameleonic capacity of $[3,3'\text{-Co}(1,2\text{-C}_2\text{B}_9\text{H}_{11})_2]\text{-}$ in coordination. Generation of the highly uncommon S(thioether)–Na bond. *Organometallics*, **2003**, 22, 3414-3423.
- (13) Llop, J.; Masalles, C.; Viñas, C.; Teixidor, F.; Sillanpää, R.; Kivekäs, R. The $[3,3'\text{-Co}(1,2\text{-C}_2\text{B}_9\text{H}_{11})_2]\text{-}$ anion as a platform for new materials: synthesis of its functionalized monosubstituted derivatives incorporating synthons for conducting organic polymers. *Dalton Trans.* **2003**, 556-561.
- (14) (a) Sivaev, I. B.; Starikova, Z. A.; Sjöberg, S.; Bregadze, V. I. Synthesis of functional derivatives of the $[3,3'\text{-Co}(1,2\text{-C}_2\text{B}_9\text{H}_{11})_2]\text{-}$ anion. *J. Organomet. Chem.* **2002**, 649, 1-8. (b) Sivaev, I. B.; Sjöberg, S.; Bregadze, V. I., International Conference Organometallic Compounds - Materials of the Next Century, Nizhny Novgorod, Russia, May 29-June 2, **2000**.
- (15) Plešek, J.; Grüner, B.; Heřmánek, S.; Báča, J.; Mareček, V.; Jänchenová, J.; Lhotský, A.; Holub, K.; Selucký, P.; Rais, J.; Císařová I.; Čáslavský, J. Synthesis of functionalized cobaltacarboranes based on the $\text{closo}-[(1,2\text{-C}_2\text{B}_9\text{H}_{11})_2\text{-}3,3'\text{-Co}]\text{-}$ ion bearing polydentate ligands for separation of M^{3+} cations from nuclear waste solutions. Electrochemical and liquid–liquid extraction study of selective transfer of M^{3+} metal cations to an organic phase. Molecular structure of the $\text{closo}-[(8\text{-(}2\text{-CH}_3\text{O-C}_6\text{H}_4\text{-O)-(CH}_2\text{CH}_2\text{O)}_2\text{-}1,2\text{-C}_2\text{B}_9\text{H}_{10}\text{)-(}1',2'\text{-C}_2\text{B}_9\text{H}_{11}\text{)-}3,3'\text{-Co}]\text{Na}$ determined by X-ray diffraction analysis. *Polyhedron* **2002**, 21, 975-986.

-
- (16) Grüner, B.; Plešek, J.; Báča, J.; Císařová, I.; Dozol, J.-F.; Rouquette, H.; Viñas, C.; Selucký, P.; Rais, J. Cobalt bis(dicarbollide) ions with covalently bonded CMPO groups as selective extraction agents for lanthanide and actinide cations from highly acidic nuclear waste solutions. *New J. Chem.* **2002**, *26*, 1519-1527.
- (17) (a) Grüner, B.; Mikulašek, L.; Báča, J.; Císařová, I.; Böhmer, V.; Danila, C.; Reinoso-Garcia, M. M.; Verboom, W.; Reinhoudt, D. N.; Casnati, A.; Ungaro, R. Cobalt Bis(dicarbollides)(1-) Covalently Attached to the Calix[4]arene Platform: The First Combination of Organic Bowl- Shaped Matrices and Inorganic Metallaborane Cluster Anions. *Eur. J. Org. Chem.* **2005**, 2022-2039. (b) Mikulašek, L.; Grüner, B.; Danila, C.; Böhmer, V.; Časlavský, J.; Selucky, P. Synergistic effect of ligating and ionic functions, prearranged on a calix[4]arene. *Chem. Commun.* **2006**, 4001-4003.
- (18) (a) Olejniczak, A. B.; Plešek, J.; Kříž, O.; Lesnikowski, Z. J. A Nucleoside conjugate containing a metallacarborane group and its incorporation into a DNA oligonucleotide. *Angew. Chem., Int. Ed.* **2003**, *42*, 5740-5743. (b) Lesnikowski, Z. J.; Paradowska, E.; Olejniczak, A. B.; Studzinska, M.; Seekamp, P.; Schüßler, U.; Gabel, D.; Schinazi, R. F.; Plešek, J. Towards new boron carriers for boron neutron capture therapy: metallacarboranes and their nucleoside conjugates. *Bioorg. Med. Chem.* **2005**, *13*, 4168-4175. (c) Olejniczak, A. B.; Plešek, J.; Lesnikowski, Z. J. Nucleoside–metallacarborane conjugates for base-specific metal labeling of DNA. *Chem. Eur. J.* **2007**, *13*, 311-318.
- (19) (a) Farràs, P.; Šícha, V.; Štíbr, B.; Teixidor, F.; Grüner, B.; Viñas, C. Syntheses of C-substituted icosahedral dicarbaboranes bearing the 8-dioxane-cobalt bisdicarbollide moiety. *J. Organomet. Chem.* **2009**, *694*, 1599-1601; (b) Farràs, P.; Cioran, A.M.; Šícha, V.; Teixidor, F.; Štíbr, B.; Grüner, B.; Viñas, C. Toward the synthesis of high boron content polyanionic multicluster macromolecules. *Inorg. Chem.* **2009**, *48*, 8210–8219.
- (20) (a) Hawthorne, M. F. The role of chemistry in the development of boron neutron capture therapy of cancer. *Angew. Chem. Int. Ed.*, **1993**, *32*, 950-984. (b) Soloway, A.H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I.M.; Wilson, J. G. The chemistry of neutron capture therapy. *Chem. Rev.*, **1998**, *98*, 1515-1562. (c) Valliant, J. F.; Guenther, K. J.; King, A. S.; Morel, P.; Schaffer, P.; Sogbein, O. O.; Stephenson, K. A. The medicinal chemistry of carboranes. *Coord. Chem. Rev.*, **2002**, *232*, 173-230. (d) Sivaev, I. B.; Bregadze, V.; Sjöberg, S. in *Research and*

Development in Neutron Capture Therapy, ed. W. Sauerwein, R. Moss and A. Wittig, Monduzzi Editore S.p.A., Bologna, **2002**, pp. 19–23. (e) *Boron Science, New Technologies and Applications*, Ed. N. S. Hosmane, CRC Press: Boca Raton, FL, USA, **2012**. (f) *Handbook of Boron Chemistry in Organometallics, Catalysis, Materials and Medicine*, Volume 4: Boron in Medicine, Eds. N. S. Hosmane, R. Eagling, World Science Publishers, New Jersey **2018**. (g) *Boron-Based Compounds. Potential and Emerging applications in Medicine*, Eds. E. Hey-Hawkins and C. Viñas Teixidor, John Wiley & Sons Ltd, Chichester, UK.

- (21) (a) Viñas, C. The uniqueness of boron as a novel challenging element for drugs in pharmacology, medicine and for smart biomaterials. *Future Med. Chem.* **2013**, 5 (6), 617-619. (b) Chellan, P.; Sadler, J. P. The elements of life and medicines. *Phil. Trans. R. Soc. A.* **2015**, 373, 20140182. (c) Pache, W.; Zähler, H. Metabolic products of microorganisms. *Arch. Mikrobiol.* **1969**, 67, 156–165. (d) Soriano-Ursúa, M. A.; Das, B. C.; Trujillo-Ferrara, J. G. Boron-containing compounds: chemico-biological properties and expanding medicinal potential in prevention, diagnosis and therapy. *Expert. Opin. Ther. Pat.* **2014**, 24, 485–500. (e) Ciaravino, V.; Plattner, J.; Chanda, S. An assessment of the genetic toxicology of novel boron- containing therapeutic agents. *Environ. Mol. Mutagen.* **2013**, 54, 338–346.
- (22) (a) Totani, T.; Aono, K.; Yamamoto, K.; Tawara, K. Synthesis and in Vitro Antimicrobial Property of *o*-Carborane Derivatives. *J. Med. Chem.* **1981**, 24, 1492-1499. (b) Li, Sh.; Wang, Zh.; Wei, Y.; Wu, Ch.; Gao, Sh.; Jiang, H.; Zhao, X.; Yan, H.; Wang, X. Antimicrobial activity of a ferrocene-substituted carborane derivative targeting multidrug-resistant infection. *Biomaterials*, **2013**, 34, 902-911.
- (23) Popova, T.; Zaulet, A.; Teixidor, F.; Alexandrova, R.; Viñas, C. Investigations on antimicrobial activity of cobaltabisdicarbollides. *J. Organomet. Chem.* **2013**, 747, 229-234.
- (24) Zheng, Y.; Liu, W. W.; Chen, Y.; Jiang, H.; Yan, H.; Kosenko, I.; Chekulaeva, L.; Sivaev, I.; Bregadze, V.; Wang, X. M. A Highly potent antibacterial agent targeting methicillin-resistant staphylococcus aureus based on cobalt bis(1,2-dicarbollide) alkoxy derivative. *Organometallics* **2017**, 36 (18), 3484-3490.
- (25) Kvasničková E.; Masák, J.; Čejka, J.; Matátková, O.; Šícha, V. Preparation, characterization, and the selective antimicrobial activity of N-alkylammonium 8-diethyleneglycol cobalt bis-dicarbollide derivatives. *J. Organomet. Chem.* **2017**, 827, 23-31.

-
- (26) Vaňková E.; Lokocová, K.; Matátková, O.; Křížová, I.; Masák, J.; Grüner, B.; Kaule, P.; Čermák, J.; Šícha, V. Cobalt bis-dicarbollide and its ammonium derivatives are effective antimicrobial and antibiofilm agents *J. Organomet. Chem.* **2019**, 899, 120891.
- (27) (a) Baker, S. J.; Akama, T.; Zhang, Y. K.; Sauro, V.; Pandit, C.; Singh, R.; Kully, M.; Khan, J.; Plattner, J.; Benkovic, S. J.; Lee, V.; Maples, K. R. Identification of a novel boron-containing antibacterial agent (AN0128) with anti-inflammatory activity, for the potential treatment of cutaneous diseases. *Bioorg. Med. Chem. Lett.* **2006**, 16, 5963-5967. (b) Mendes, R. E.; Alley, M. R. K.; Sader, H. S.; Biedenbach, D. J.; Jones, R. N. Potency and spectrum of activity of AN3365, a novel boron-containing protein synthesis inhibitor, tested against clinical isolates of *enterobacteriaceae* and nonfermentative Gram-negative bacilli. *Agents Chemother.* **2013**, 57 (6), 2849-2857. (c) Gorovoy, A. S.; Gozhina, O. V.; Svendsen, J. S.; Domorad, A. A.; Tetz, G. V.; Tetz, V. V.; Lejon, T. Drugs. Boron-containing peptidomimetics – A novel class of selective anti-tubercular drugs. *Chem. Biol. Drug. Des.* **2013**, 81, 408-413.
- (28) Kahl, S.B.; Kasar, R.A. *J. Am. Chem. Soc.*, **1996**, 118, 1223-1224. Simple, High-Yield Synthesis of Polyhedral Carborane Aminoacids.
- (29) Hawthorne, M. F.; Young, D. C.; Andrews, T. D.; Howe, D. V.; Pilling, R. L.; Pitts, A. D.; Reintjes, M.; Warren, L. F., Jr.; Wegner, P.A. π -Dicarbollyl Derivatives of the Transition Metals. Metallocene Analogs. *J. Am. Chem. Soc.* **1968**, 90, 879-896.
- (30) Farràs, P.; Teixidor, F.; Kivekäs, R.; Sillanpää, R.; Viñas, C.; Grüner, B.; Cisarova, I. Metallocarboranes as building blocks for polyanionic polyarmed aryl-ether materials. *Inorg. Chem.* **2008**, 47(20), 9497-9508.
- (31) Darfeuille-Michaud, A.; Neut, C.; Barnich, N.; Lederman, E.; Di Martino, P.; Desreumaux, P.; Gambiez, L.; Joly, B.; Cortot, A.; Colombel, J.F. Presence of adherent *Escherichia coli* strains in ileal mucosa of patients with Crohn's disease. *Gastroenterology*, **1998**, 115(6), 1405-1413.

'For TOC Only'

